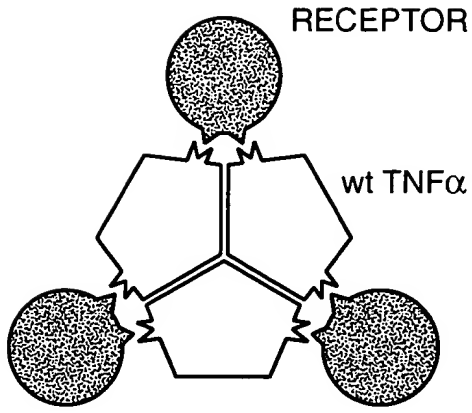


FIG._1A

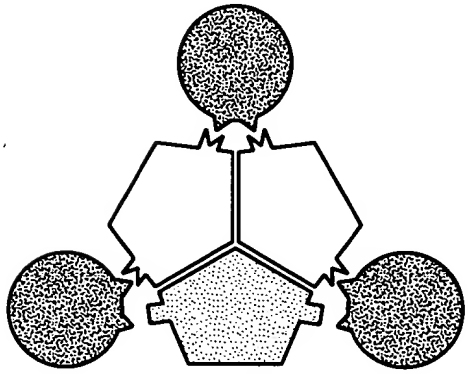


FIG._1B

TNF-TNFR TRIMER COMPLEX

TOP VIEW

SIDE VIEW

FIG._2

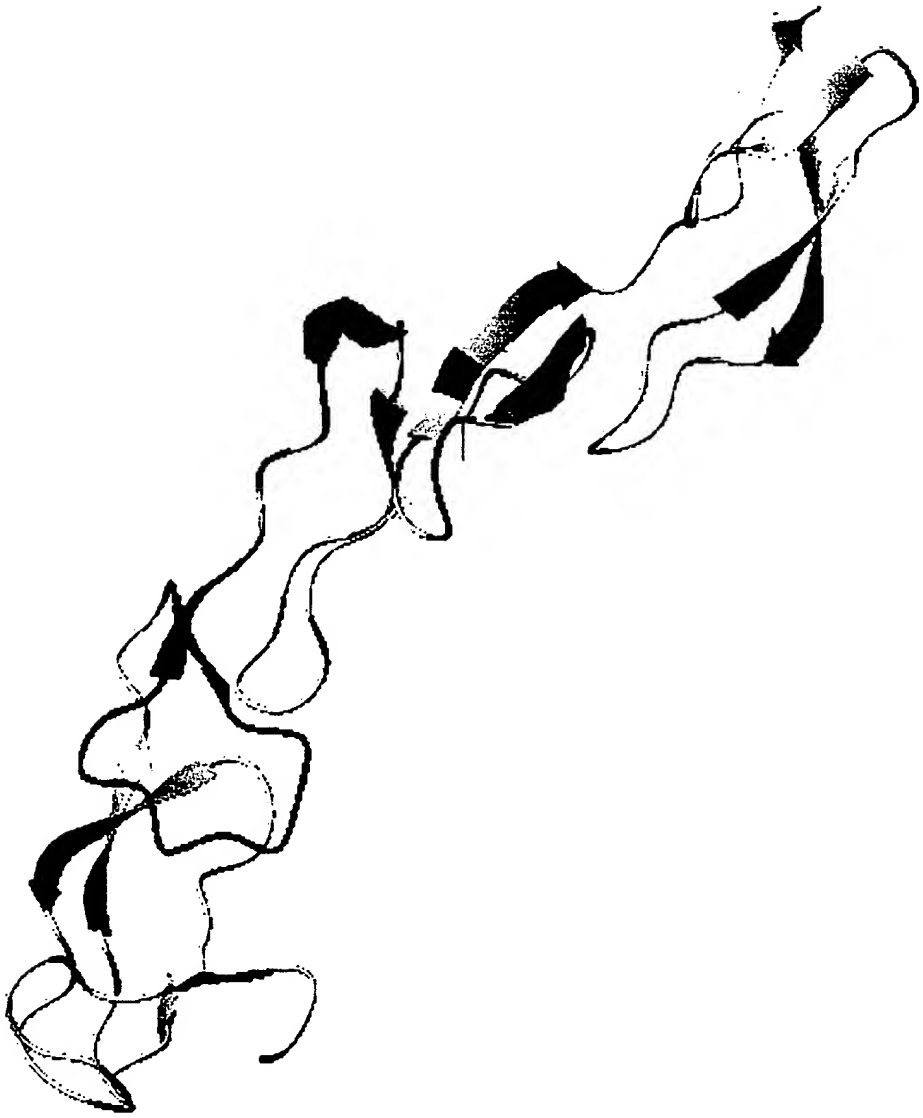


FIG._3

TNFα BINDING SITES

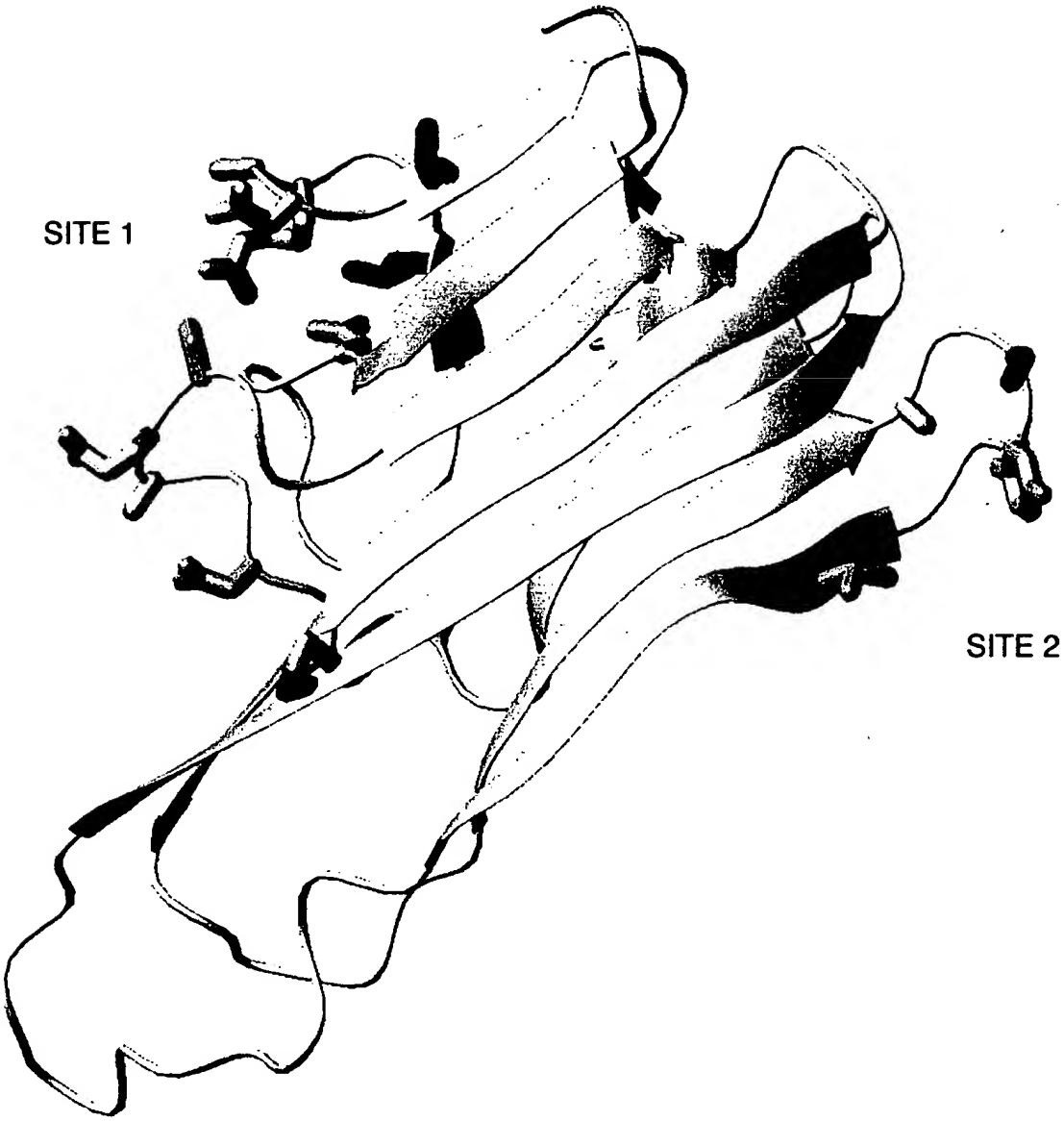


FIG._4

TNFa TRIMER INTERFACE

FIG._5

```
1    atgcaccacc accaccacca cgtacgctcc tcctcccgca ctccgtccga caaaccggta
61   gctcacgtag tagctaaccc gcaggctgaa ggtcagctgc agtggctgaa ccgccgcgct
121  aacgctctgc tggctaacgg tgtagaactg cgcgacaacc agctggtagt accgtccgaa
181  ggtctgtacc tgatctactc ccaggtactg ttcaaaggtc agggttgtcc gtccactcac
241  gtactgctga ctcacactat ctcccgcatc gctgtatcct accagactaa agtaaacctg
301  ctgtccgcta tcaaatcccc gtgtcagcgc gaaactccgg aaggtgctga agctaaaccg
361  tggtacgaac cgatctacct gggtggtgta ttccagctgg aaaaaggtga ccgcctgtcc
421  gctgaaatca accgcccgga ctacctggac ttcgctgaat ccggtcaggt atacttcggt
481  atcatcgctc tgtga
```

FIG._6A

```
1    MHHHHHHVRS SSRTPSDKPV AHVVANPQAE GQLQWLNRRA NALLANGVEL RDNQLVVPSE

61   GLYLIYSQVL FKGQGCPSTH VLLTHTISRI AVSYQTKVNL LSAIKSPCQR ETPEGAEAKP

121  WYEPIYLGGV FQLEKGDRLS AEINRPDYLD FAESGQVYFG IIAL
```

FIG._6B

Wild-type TNF amino acid	Wild-type TNF amino acid number	Mutants created
Q	21	R
N	30	D
R	31	I, D, E
R	32	D, E, S
A	33	E
A	35	S
K	65	D, T, M, W, I, Q, S, N, V, E
G	66	Q, K
Q	67	D, W, Y, R, K, S
A	111	R, E
K	112	D, E
Y	115	Q, K, E, N, R, F, H, M, L, I, W, D, T, S
D	140	R, K
D	143	E, N, Q, S, R, K
F	144	N
A	145	R, D, K, N, H, T, Q, E, Y, M, S, F
E	146	N, K, R, S
S	147	R

ALSO MADE DOUBLE MUTANTS K65E/D143K, K65E/D143R, K65D/D143K AND K65D/D143R

FIG._7

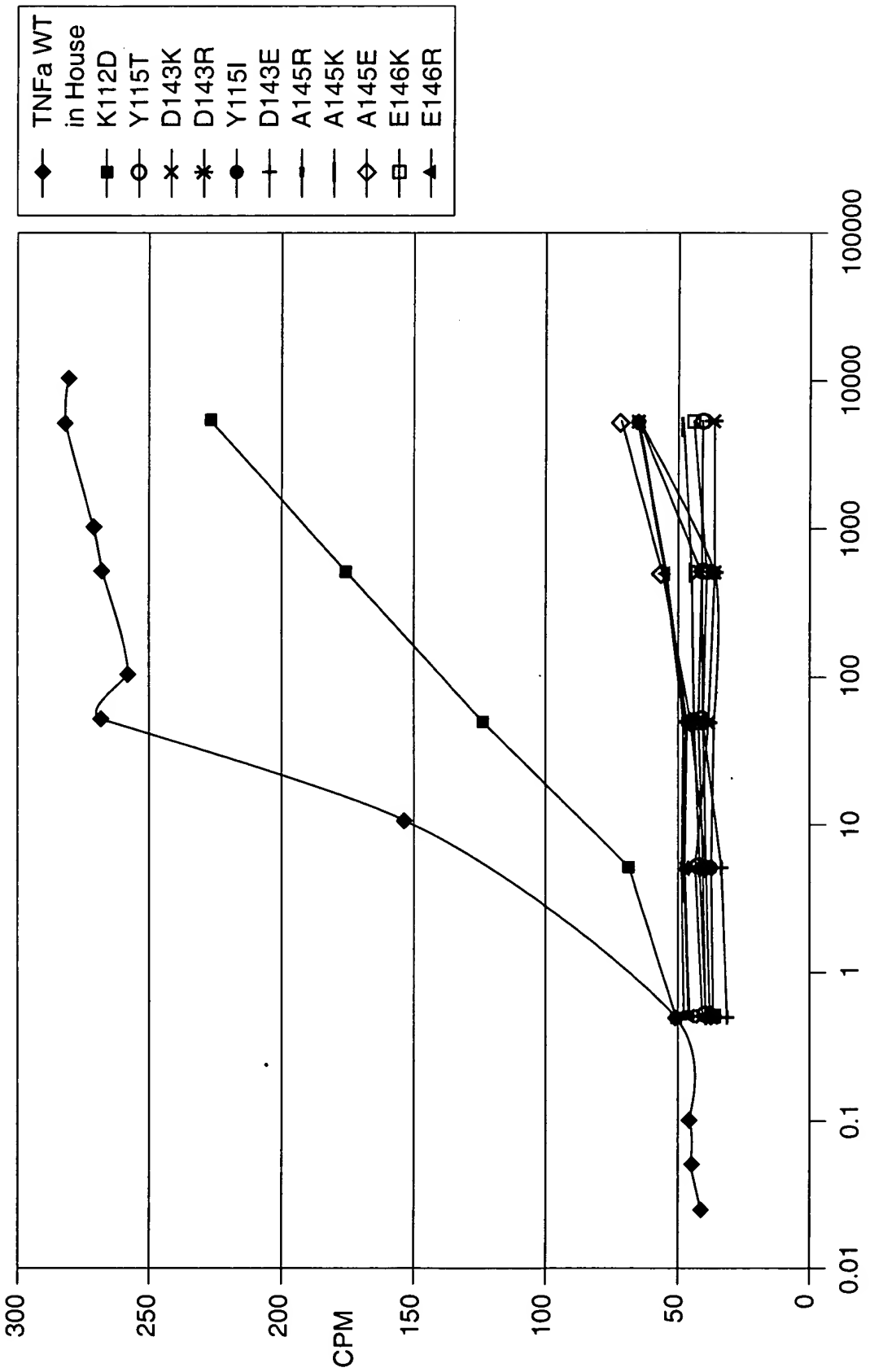

TNFa WT in House
K112D
Y115T
D143K
D143R
Y115I
D143E
A145R
A145K
A145E
E146K
E146R
CPM
300
250
200
150
100
50
0
0.01
0.1
1
10
100
1000
10000
100000
TNF-a (ng/mL)

FIG._8

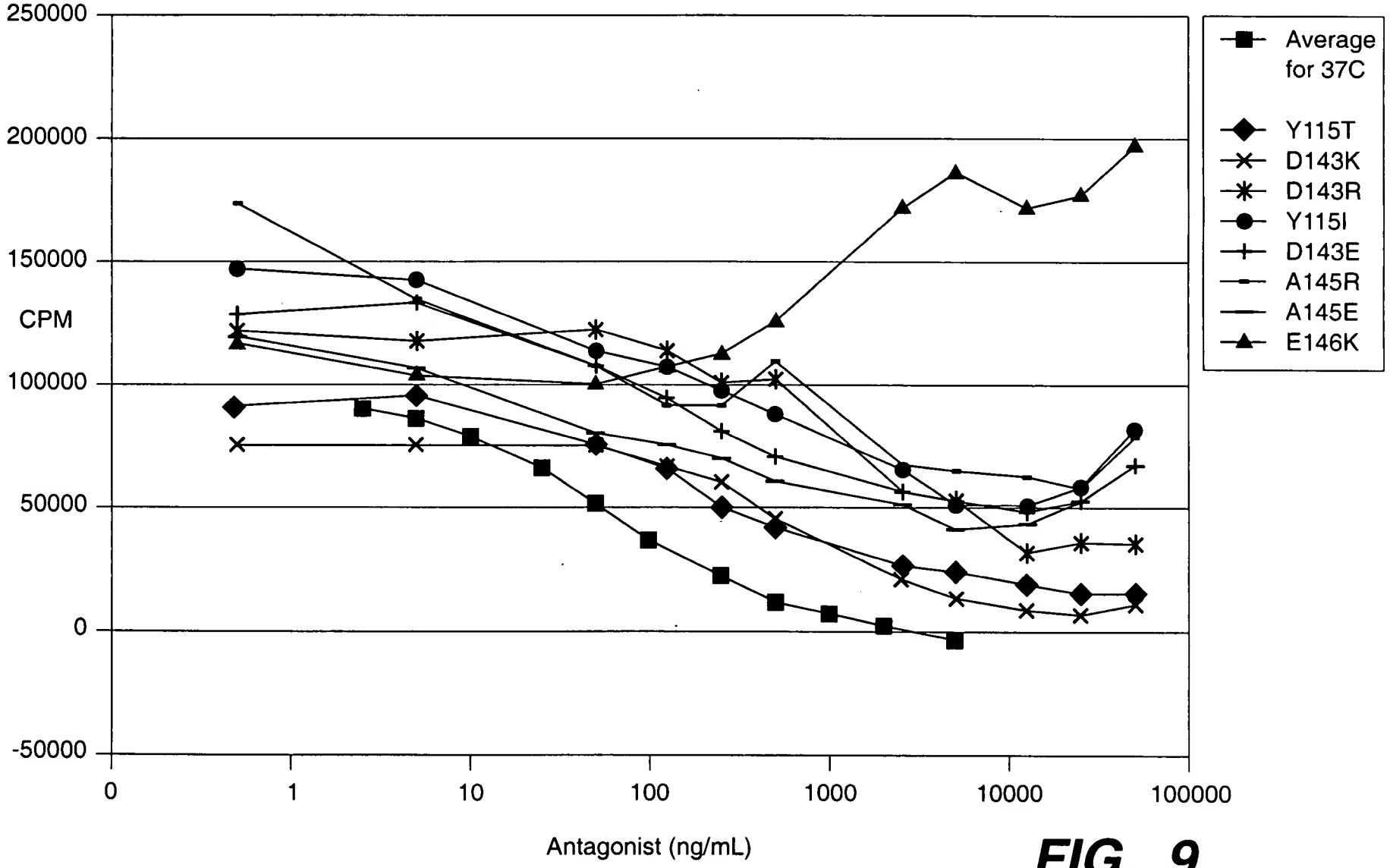

250000
200000
150000
CPM
100000
50000
0
-50000
0
1
10
100
1000
10000
100000
Antagonist (ng/mL)
Average for 37C
Y115T
D143K
D143R
Y115I
D143E
A145R
A145E
E146K

FIG._9

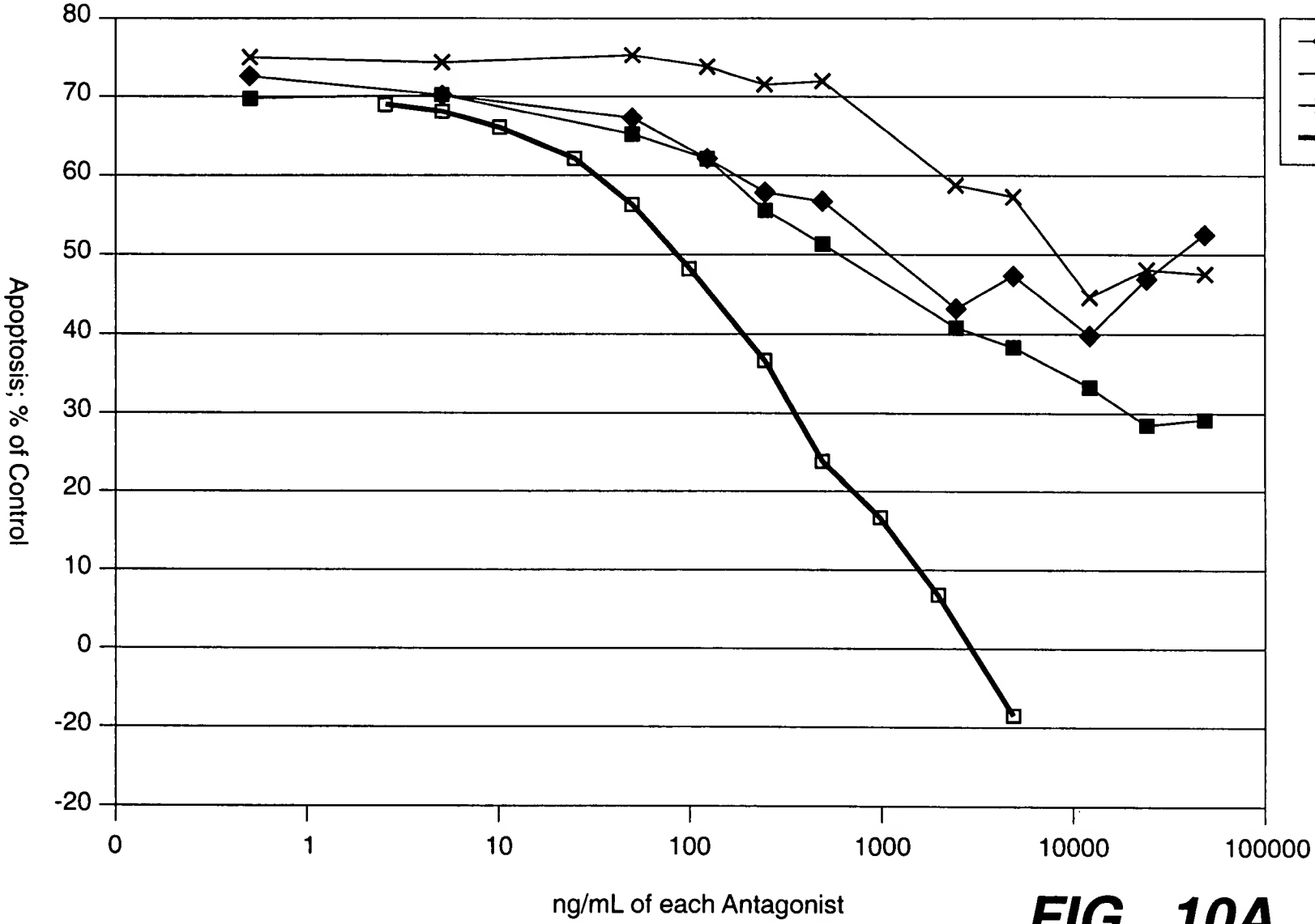

80
70
60
50
40
30
20
10
0
-20
-20
Apoptosis; % of Control
0
1
10
100
1000
10000
100000
ng/mL of each Antagonist
K112D
Y115T
D143R
STD TNF R

FIG._10A

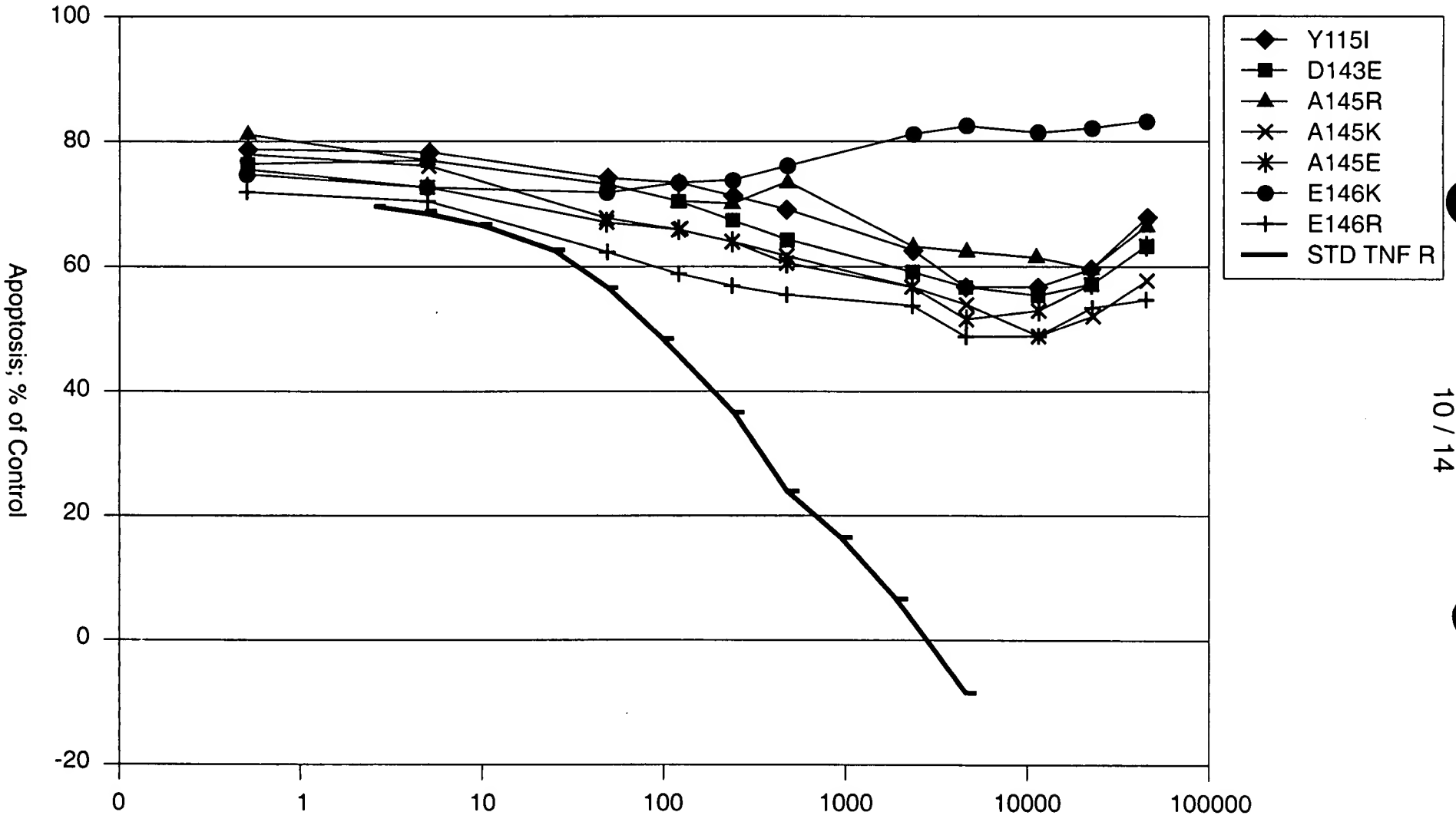

100
80
60
40
20
0
-20
Apoptosis; % of Control
0
1
10
100
1000
10000
100000
ng/mL of each Antagonist
Y115I
D143E
A145R
A145K
A145E
E146K
E146R
STD TNF R

FIG._10B

WT	PDA Relative Probability Distribution
Q21	R1000
N30	D1000
R31	I1000
R32	H1000
A33	E1000
A35	S1000
K65	R585 D146 K110 T42 H31 M27 W15 I15 Q10 S9 N9 V1
G66	Q813 K187
Q67	D623 W209 Y83 R43 K41 S1
A111	R959 E41
K112	K1000
Y115	Q230 K154 E116 N84 Y81 R72 F69 H43 M39 L36 I26 W25 D11 T8 S6
D140	D1000
L143	D680 E130 N110 Q33 S29 R12 K6
F144	F695 N305
A145	R456 D196 K124 N76 H67 T43 Q25 E9 Y1 M1 S1 F1
E146	N489 K377 R111 D12 S10 E1
S147	R1000

FIG._11

TRAF2(310-) DQDKIEALSSKVQQLERSIGLKDLAMADLEQKVLEMEA STYDG

FIG._12A

TRAF3(374-) VARNTGLLESQLSRHDQMLSVHDIRLADMDLRFQVLET ASYNG

FIG._12B

TRAF5(343-) NDQRLAVLEEETNKHDTHINIHKAQLSKNEERFKLLEG TCYNG

FIG._12C

TRAF1(225-) DRERILSLEQRVVELQQTLAQKDQALGKLEQSLRLMEE ASFDG

FIG._12D

TRAF6(309-) QDHQIRELTAKMETQSMYVSELKRTIRTLEDKVAEIEA QQCNG

FIG._12E

TRAF4(201-) ----------CALVSRQRQELQELRRELEELSV GS-DG

FIG._12F

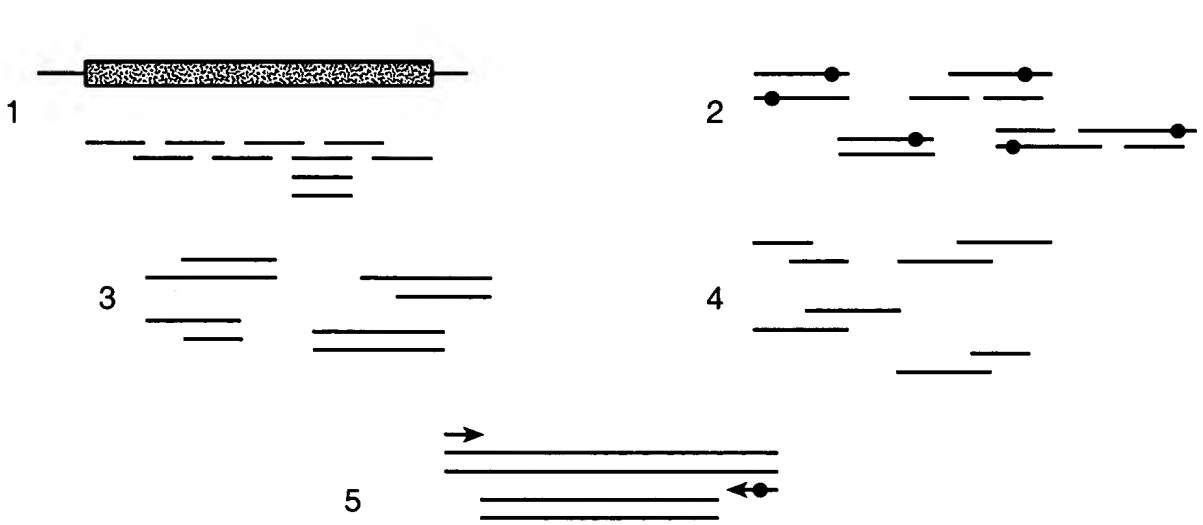


FIG._13

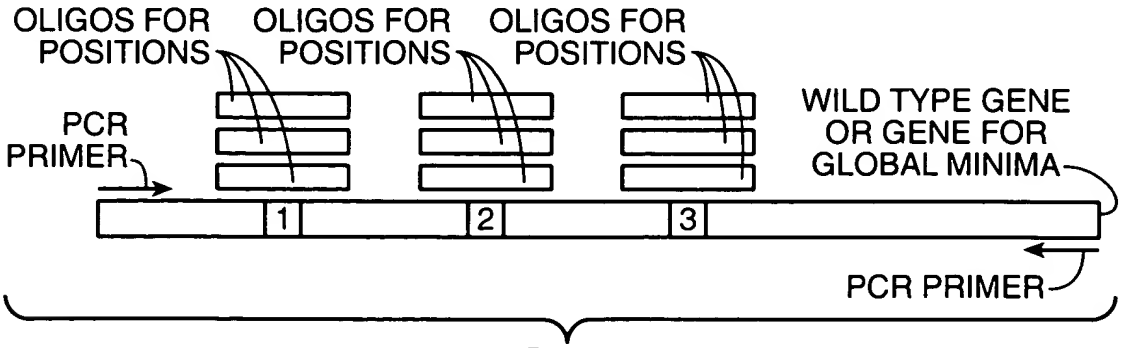
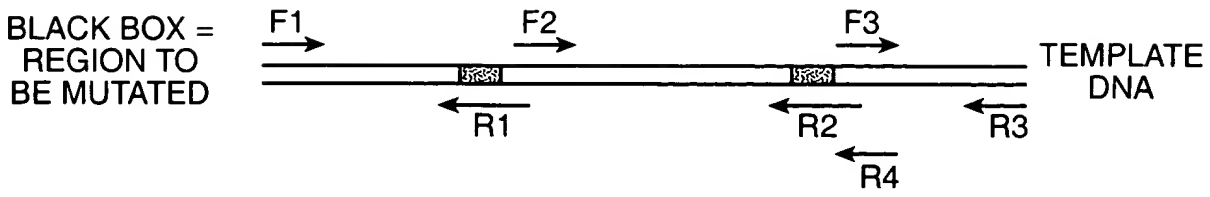


FIG._14



STEP 1: SET UP 3 PCR REACTIONS:

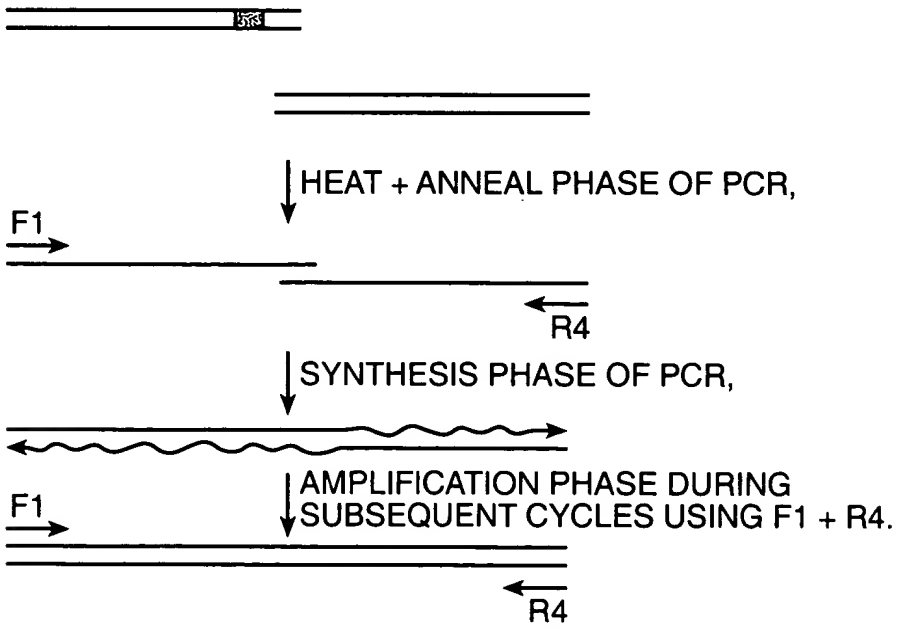
PRODUCTS:

TUBE 1:

TUBE 2:

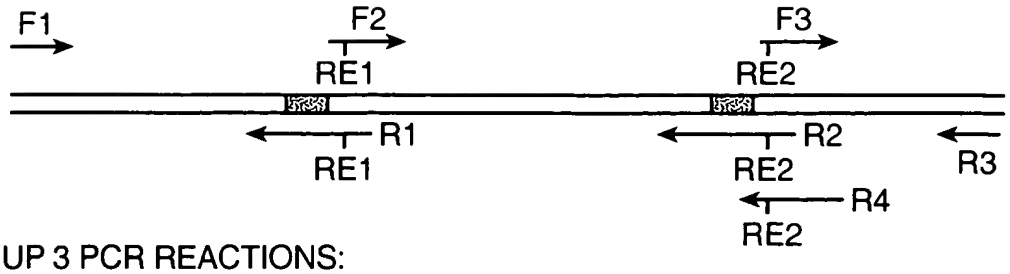
TUBE 3:

STEP 2: SET UP PCR REACTION WITH PRODUCTS OF TUBE 1 + PRODUCTS TUBE 2 + F1 + R4.



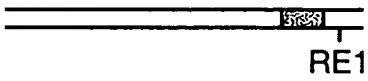
STEP 3: REPEAT STEP 2 USING PRODUCT FROM STEP 2 + PRODUCT FROM STEP 1, TUBE 3 + PRIMERS F1 + R3.

FIG._15

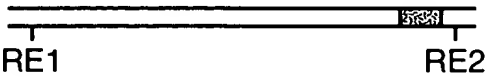


STEP 1: SET UP 3 PCR REACTIONS:

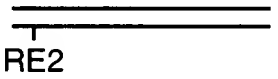
TUBE 1:



TUBE 2:

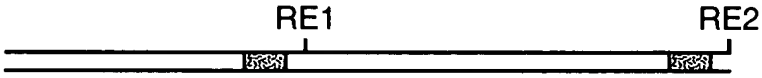


TUBE 3:

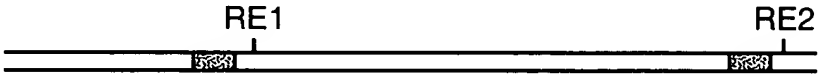


STEP 2: DIGEST PRODUCTS FROM STEP 1 WITH SUITABLE RESTRICTION ENDONUCLEASES.

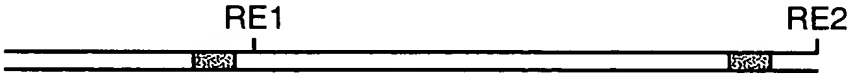
STEP 3: LIGATE DIGESTED PRODUCT FROM STEP 2, TUBE 2 WITH DIGESTED PRODUCT FROM STEP 2, TUBE 1.



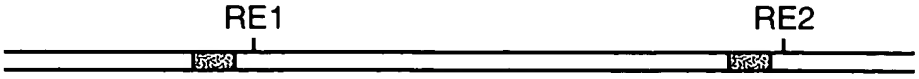
STEP 4: AMPLIFY VIA PCR LIGATED PRODUCTS OF STEP 3 WITH F1 + R4.



STEP 5: DIGEST AMPLIFIED PRODUCT OF STEP 4 WITH RESTRICTION ENDONUCLEASE #2.



STEP 6: LIGATE PRODUCT FROM STEP 5 WITH PRODUCT FROM STEP 2, TUBE 1.



STEP 7: AMPLIFY PRODUCT FROM STEP 6 WITH F1 + R3.

FIG._16

DIAGRAM 3

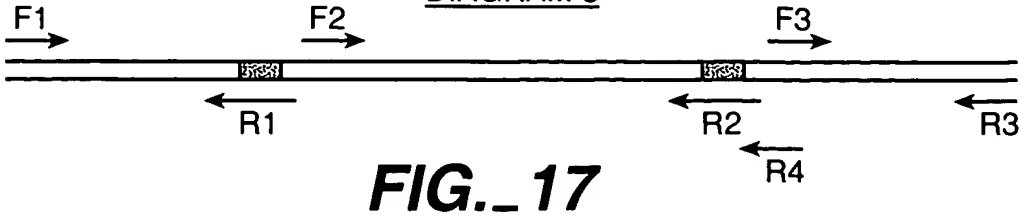


FIG._17